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STORAGE

STUDIES OF AN ACID SOIL IN ASSAM, II

BY

A. A. MEGGITT, B.Sc., F.C.S

Agricultural Chemist, Assam

AGRICULTURAL RESEARCH INSTITUTE, PUSA

PRINTED AND PUBLISHED FOR

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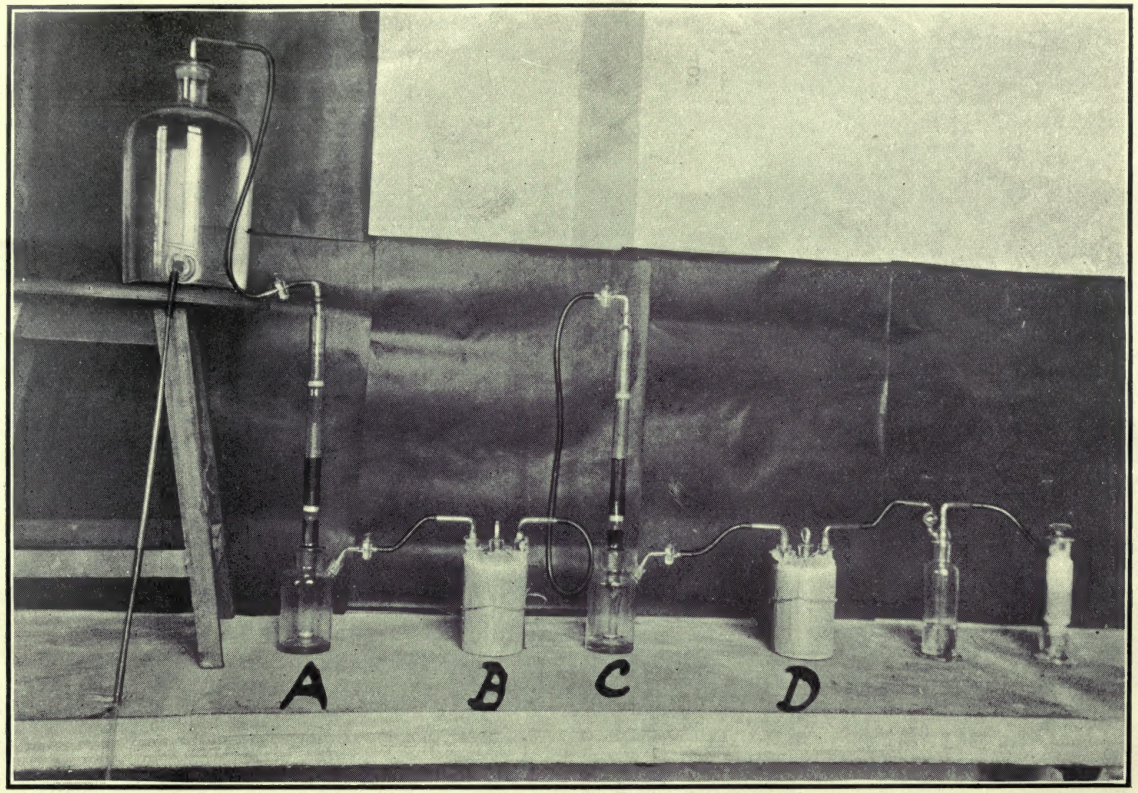

Apparatus used to study comparative Co_2 evolution from soils.

PLATE I.

STUDIES OF AN ACID SOIL IN ASSAM, II.

BY

A. A. MEGGITT, B.Sc., F.C.S.,

Agricultural Chemist, Assam.

[Received for publication on September 5, 1922.]

LABORATORY work undertaken in connection with certain field experiments on the Government Farm at Jorhat, Assam, during the past 15 years has revealed what very serious losses of soil nitrogen may take place from the cultivated surface layer of highland areas in a climate like that of Assam. The normal rainfall is of the order of 80 inches, by far the greater portion of which falls within the five months from May to September.

At the time of its acquisition the farm site was a grazing area growing only coarse grasses and low scrub jungle. Representative soil analyses will be found in the Appendix.

Some idea of the nitrogen losses may be gathered from the following :—

Area	Initial N % 0 – 6″	Final N % 0 – 6″	Percentage of initial N lost	Lb. N per acre lost from surface 6″	Remarks
Block C .. Plot IA ..	0·090 (in 1911)	0·055 (in 1921)	39 in 10 years	Some 700 lb. in 10 years	Land cultivated but uncropped and unmanured.
Block E ..	0·115 (in 1909)	0·078 (in 1921)	32 in 12 years	Some 740 lb. in 12 years	Land under sugarcane rotation. Some 750 lb. N have been added in manures in 12 years and 5 green manure crops have been ploughed in.

Similar large losses of nitrogen have been observed on the new alluvium in the Kamrup District of Assam where cultivable waste land was opened out for sugarcane.

For the purpose of obtaining the necessary soil samples a soil auger was used throughout, the number of samples taken per acre varying from 800 in one series of plots to 8,000 per acre in another series. The degree of accuracy thus obtained in sampling is shown by the following analyses of duplicate composite samples taken from four plots :—

Nitrogen percentage on dry soil.

Plot No.	Sample 1	Sample 2
1 ..	0·095	0·094
2 ..	0·090	0·090
3 ..	0·086	0·087
4 ..	0·074	0·075

Again, duplicate analyses of each sample were made by the Kjeldahl method, using 15 grm. soil, every possible refinement being introduced to reduce error alike in the digestion, distillation and final titration. In the result the difference between duplicate analyses of the individual samples very rarely exceeded 1 mg. of nitrogen per 100 grm. soil, and on the average was less than this.

In connection with experiments on the use of lime on this soil which have been in progress for the past 12 years, the figures in Table I are of interest.

It was shown in a previous Memoir (Chem. Ser., III, 9) of the Department of Agriculture in India entitled " Studies of an Acid Soil in Assam " that the primary requirement of this soil for most farm crops was a base of some sort. The use of lime has led to a very great increase in the yield of sugarcane and other crops. The plots from which the figures in Table I are derived form part of an experiment dating from 1911 to test the value of large initial lime dressings versus smaller and more frequent ones in a crop rotation. The four plots in question have not been manured in any way beyond receiving the differential lime treatment specified. The case is therefore not complicated by the question of direct nitrogen additions. The crops taken during the 10 years 1911-1921 include :—

	No. of crops
Aus paddy (*Oryza sativa*).	2
Oats (*Avena sativa*).	4
Jowar (*Andropogon Sorghum*).	5

	No. of crops
Rape (*Brassica campestris*).	2
Barley (*Hordeum vulgare*).	1
Matikalai (*Phaseolus mungo* var. *radiatus*).	1
Maize (*Zea Mays*).	1
Soybean (*Glycine hispida*).	1
Gram (*Cicer arietinum*).	1

Beyond carrying a small paddy crop, plot IA has returned no crop at all; the absence of lime prevents this. The three limed plots have throughout carried crops, naturally small in the absence of other manuring, of all varieties sown. The removals of nitrogen in grain are given in the table, but are only available for the six years 1911–1917.

It is necessary to emphasize here, for a proper understanding of the table, that up to the end of 1919 the plots were regularly cropped twice a year; in 1920 it was decided to let the plots lie fallow, the effect of which on soil nitrogen loss was very marked as will presently appear.

For the period 1911–1919 it will be seen that although from each of the plots Nos. 2A, 3A and 4A at least 100 lb. nitrogen per acre had been removed in grain and straw, nevertheless these three limed plots lost in the eight years less of their initial nitrogen than did the unlimed plot IA, and that the loss was least in the case of plots 3A and 4A which had the heavier initial lime dressings and which carried the heavier crops during the first five years of the experiment. In other words the plots which carried crops lost appreciably less nitrogen than did the one which failed to crop, and the heavier the crops the less the loss. Other work, to be presently described, has proved that limed plots lose a good deal more nitrogen in a given time than do unlimed ones provided both are uncropped. This fact would still further improve the argument in favour of the preventive action of growing crops on soil nitrogen loss as indicated by the above results. This is again emphasized by the effect of the fallow year 1920 referred to above. Thus we find that for the period 1919–1921, embracing the fallow year, the limed plots 2A, 3A and 4A lost a considerably greater amount of nitrogen than did plot IA. So much so that it is true to say that this one fallow year in itself sufficed to negative almost completely, as the figures show, the conservative action of the cropping of the whole previous eight years, the limed plots on the average losing more than three times as much nitrogen during the fallow as the unlimed plot.

The position of these four plots just before the fallow in regard to lime requirement, nitrogen in surface six inches, and relative cropping power as

indicated by a crop of *jowar* taken just previous to the fallow year, is shown below :—

Plot No.	Nitrogen in surface six inches	Lime requirement (Parts CaO per million)	Relative cropping power
	Per cent.		
1A	0·058	690	0
2A	0·061	nil	100
3A	0·067	280	92
4A	0·066	340	61

It will be seen that in regard to cropping power the nitrogen content of these soils plays a very insignificant part as compared with the lime requirement.

Considered in conjunction with the figures in Table I it appears then that during the fallow period the nitrogen loss varied inversely as the lime requirement.

TABLE I.

Plot	Treatment	Soil nitrogen percentage in 1911 0 – 6″	Soil nitrogen percentage in 1919 0 – 6″	Soil nitrogen percentage in 1921 0 – 6″	Percentage of initial nitrogen lost in eight years 1911–1919	Percentage of initial nitrogen lost in ten years 1911–1921	Nitrogen, lb. per acre, lost from surface six inches for eight years 1911–1919	Nitrogen, lb. per acre, lost from surface six inches for ten years 1911–1921	Nitrogen, lb. per acre, removed in grain for six years 1911–1917	Remarks
1	2	3	4	5	6	7	8	9	10	11
							lb.	lb.	lb.	
1A	No lime	0·090	0·058	0·055	35	39	640	700	4	Cultivated regularly but uncropped as no crops grew in absence of lime.
2A	Lime total 4,800 lb., *i.e.*, 800 lb. annually for six years from 1911–1916 ..	0·085	0·061	0·050	28	41	480	700	70	Cropped regularly twice a year except in 1920 which was a fallow year.
3A	Lime total 4,800 lb., *i.e.*, 2,400 lb. in 1911 and again in 1914	0·091	0·067	0·056	26	38	480	700	80	Ditto
4A	Lime total 4,800 lb., in one initial dressing in 1911..	0·089	0·066	0·058	26	35	460	620	82·5	Ditto

The very marked influence of lime on the rate of nitrification in this soil is well shown in Fig. 1. It will be seen that the nitrate formation in 66 days was in the case of the limed soil twice that in the case of unlimed soil.

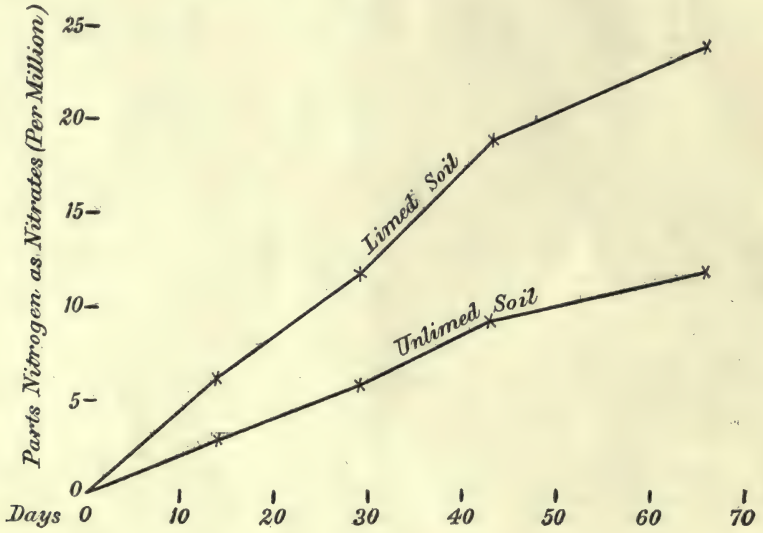

FIG. 1.
Nitrification in limed and unlimed soil.

Both cultures during the incubation period were maintained side by side at the same temperature 28°—30°C. and at a moisture content of 16·7 per cent.

The more rapid loss of soil nitrogen therefore from the limed plots during the fallow may probably be ascribed chiefly to increased loss of nitrates in drainage waters. In the absence of a lysimeter it was attempted to settle this point in another way, taking advantage of the well-known action of sugar in preventing the accumulation of nitrates; and though, owing to the later ascertained fact that this soil assimilates nitrogen from the air asymbiotically in the presence of lime and sugar, the results in regard to the increased loss of nitrogen as nitrates as a result of liming are indefinite, they are sufficiently interesting to describe.

Four small field plots, $\frac{1}{400}$ acre each, were treated as follows :—

Plot 29 E. Untreated.

,, 29 W. Ground limestone to supply 300 per cent. of lime requirement to depth of four inches.

,, 40 E. Ground limestone as above, plus periodical dressings of sugar.

,, 40 W. Ground limestone as above, plus sugar as 40 E, plus sodium phosphate.

Sugar was applied at intervals of a few weeks to plots 40 E and 40 W, as soil and weather conditions permitted, at the rate of 0·045 per cent. of the weight of the surface four inches of soil.

Sodium phosphate was applied at the rate of 25 lb. P_2O_5 per acre on two occasions to plot 40 W.

All plots were cultivated with the Planet Jnr. cultivator frequently and never more than four inches deep, and were uncropped, being kept perfectly clean even of weeds.

The plots were sampled periodically 0—4″ at the rate of 8,000 borings per acre. The surface soil on these plots was shallow but nowhere less than five inches deep, but varied considerably in depth. On this account it was decided to limit cultivation and sampling to a depth of four inches. Sampling to six inches deep would have included a little sub-soil in some cases which would have vitiated the results.

The fall in nitrogen percentage of the surface four inches is shown graphically in Fig. 2 for the period August 1919 to November 1921.

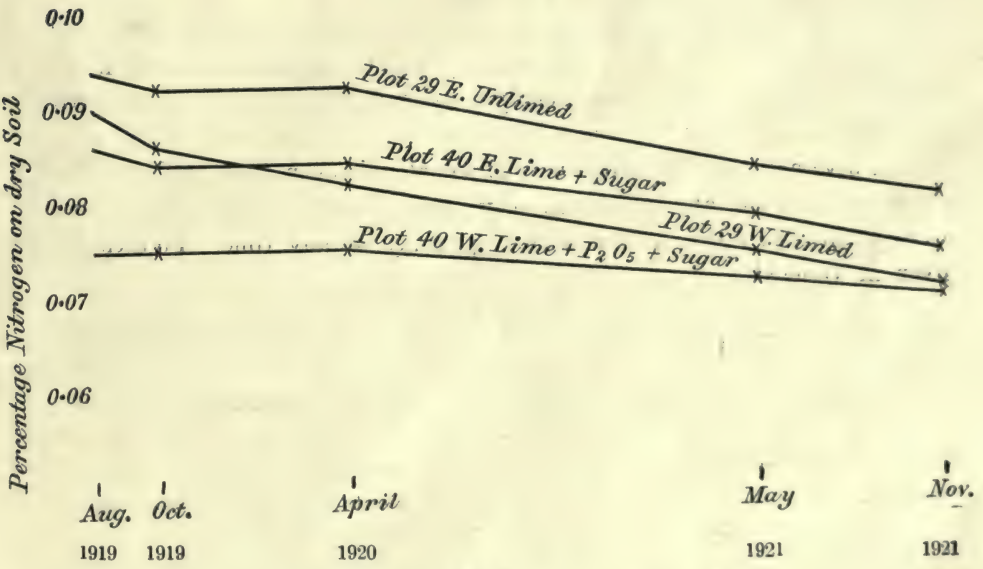

FIG. 2.

Fall in nitrogen of surface 4 inches of soil of variously treated plots.

It will be seen that the plots differed in initial nitrogen percentage and this is unfortunate as it may have influenced the extent of loss in some measure. Perhaps the fairest estimate of relative nitrogen loss will be gained

from a statement of the percentage of initial nitrogen lost for the whole period under review :—

Plot No.	Percentage of initial nitrogen lost
29 E	14·9
29 W	22·2
40 E	13·9
40 W	8·0

The loss in the case of the sugared plots represents the algebraic sum of the actual loss by leaching, etc., and any gain of nitrogen by asymbiotic assimilation.

Briefly it will be seen that, with the exception of the limed plot 29 W, there was no loss of nitrogen during the drier and colder months October to April. At this period the sugar used on the limed plots 40 E and 40 W sufficed to prevent the loss occasioned by lime alone.

For the whole period, lime alone increased the loss considerably over the unlimed plot. This result was reversed and the apparent loss was much reduced when sugar, and more specially sugar and phosphoric acid, was used as well as lime.

On several occasions estimations of nitrate nitrogen were made on the soil samples of the above plots as they came from the field. Qualitative tests for nitrites failed to reveal their presence at any time. Figures are given in Table II.

TABLE II.

Date	Plot	Nitrogen as nitrates. Parts per million	Remarks
August 12, 1919 ..	All four	1·2	Previous to the addition of any thing to the plots. Weather wet.
October 30, 1919 ..	29 E	7·2	Rains just ended. Soil moisture good. Sugar applied to 40 E and W on October 15.
	29 W	9·9	
	40 E	6·0	
	40 W	7·2	
July 1, 1921 ..	29 E	0·4	Plots 40 E and W last sugared on June 11.
	29 W	0·8	
	40 E	2·8	
	40 W	2·8	

TABLE II—*contd.*

Date	Plot	Nitrogen as nitrates. Parts per million	Remarks
July 19, 1921 ..	40 W	0·0	Last sugared on July 12.
July 21, 1921 ..	29 E	0·8	
	29 W	1·2	
August 18, 1921 ..	29 W	2·0	40 E. last sugared on July 12.
	40 E	3·6	
September 6, 1921 ..	40 E	2·4	Last sugared on August 30.
November 7, 1921 ..	40 E	1·2	40 E and W last sugared on November 1. Last rain on October 29.
	40 W	1·2	
	29 E	3·6	
	29 W	4·4	
November 17, 1921 ..	40 E	2·0	Last sugared on November 9.
	40 W	1·2	
November 19, 1921 ..	40 E	Trace	Last sugared on November 17, after sampling on that date. No rain since October 29.
	40 W	Trace	

N.B. Owing to absence on leave during the greater part of 1920, no samples were taken between April 1920 and May 1921. However, sugar was regularly applied to Plots 40 E and W during this period, the plots being kept cultivated and clean.

It will be observed that sugar was too infrequently applied and insufficient in amount to prevent all nitrification in those plots which received it. More specially during the rainy period it would appear that the nitrate determination was in most cases too long delayed after sugaring to note the immediate effect of the latter. With the cessation of the rains the reduction of nitrate by sugar is clear from the determinations made on November 7, 1921, but more specially on November 17 and 19, 1921. In the latter case the sugar added on November 17 almost entirely eliminated all nitrates within two days.

Quite interesting is the apparent fillip given to nitrification after the sugar is removed from the soil, indicated by the figures of July 1, 1921, and August 18, 1921.

This was very strongly marked in laboratory soil cultures in several cases. A good instance of this is shown graphically in Fig. 3. Cultures I and II consisted of equal weights of a sample of limed soil, kept at 16·7 per cent. moisture side by side at the same temperature 28°–30°C. To Culture I

nothing was added, while with Culture II one per cent. of saccharose was mixed. At the end of 14 days sugar was found to be absent by the d-naphthol test

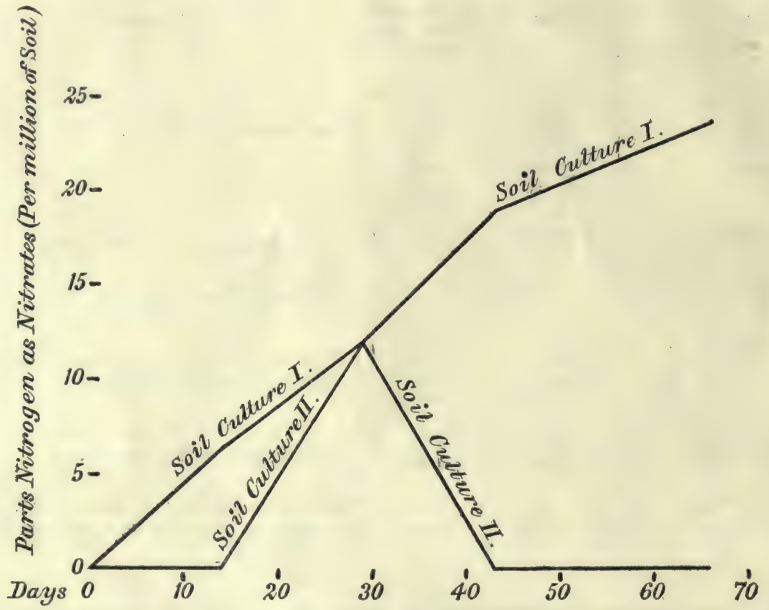

FIG. 3.

in Culture II which at this time showed an entire absence of nitrates, though Culture I contained 6·4 parts nitrate nitrogen per million. No further sugar was used at this point, and at the end of 29 days both cultures showed an identical nitrate nitrogen content of 12 parts per million. Thus in the second period of 15 days Culture II had developed as much nitrate as did Culture I in 29 days. At this point a further addition of sugar one per cent. was made to Culture II. At the end of 14 days it was found that the sugar had destroyed the whole of the nitrates in Culture II, while Culture I had advanced to 19 parts per million. One further addition of sugar was made to Culture II, and at the end of 66 days it still contained no nitrate, while Culture I showed 24 parts per million. It would appear then that sugar not only prevents the accumulation of nitrates but also destroys nitrates already formed, and so long as the merest traces of sugar persist no nitrates can be detected. With the total disappearance of sugar nitrification appears to proceed immediately at a greatly enhanced rate, which suggests either an increased activity of the organisms responsible for nitrification—a theory hard perhaps to reconcile with the immediately preceding severe repression whilst sugar is present—

or the accumulation under the influence of sugar of very readily nitrifiable material. The possibility, I think, cannot be excluded that nitrification does actually take place, and is perhaps stimulated in the presence of sugar, the nitrates being broken down as fast as formed in the presence of excess of sugar. This might serve to explain the observed enhanced rate of nitrification immediately following the final disappearance of the sugar. Nitrites could not be detected in water extracts of these two cultures at any time.

There remains the possibility of the accumulation of salts of ammonia in the presence of sugar. No evidence, however, in favour of this was found. A soil culture which had been sugared periodically over a period of eight months showed no ammonia nitrogen at all. Many other cultures confirmed this.

It was sought to determine, if possible, what happens when already accumulated nitrates are destroyed by addition of sugar, as was proved in Culture II. Attendant circumstances and conditions do not point to denitrification as ordinarily understood. Though some denitrifying bacteria can apparently operate with a limited supply of oxygen, more thorough aeration prevents it. These cultures were thoroughly aerated. The term "denitrification" as ordinarily understood includes (*a*) the reduction of nitrates to nitrites and ammonia and (*b*) reduction of nitrates to nitrites and of these to gaseous nitrogen. As already pointed out no nitrites were detectable by Metaphenylene diamine at any time and no ammonia was formed. To determine what had happened, a sample of soil was taken, the nitrogen as ammonia and by Kjeldahl being separately determined at once in duplicate. To 2,000 grm. of this soil were then added 1·5 grm. potassium nitrate, the whole very thoroughly mixed, and the nitrate nitrogen determined. Sugar at the rate of 1 per cent. was then added, and the mixture was left for a time, tests being made for sugar every few days and the moisture being kept at 15·5 per cent. At the end of 7 days sugar was completely absent, and nitrates had totally disappeared. The mixture was now Kjeldahled again, and the ammonia nitrogen also determined, with the following results, calculated to dry soil :—

Date	Nitrogen by Kjeldahl	Nitrogen as ammonia	Nitrogen as nitrates (parts per million)	Total nitrogen including that of nitrates
	Per cent.	Per cent.		Per cent.
At commencement ..	0·087	0·000	132	0·100
After seven days ..	0·097	0·000	nil	0·097

Thus while some assimilation of nitrogen may have been occasioned by the sugar added, it would appear that the greater part of the nitrate nitrogen reappears in an organic form.

To further test this point and to eliminate any error due to nitrogen fixation, two more cultures were set up as follows :—

Culture A. 1,000 grm. soil.
1 per cent. sugar.
No nitrate added.
Moisture 16 per cent.

Culture B. 1,000 grm. soil.
1 per cent. sugar.
0·75 grm. Pot. nitrate.
Moisture 16 per cent.

If the sugar caused any fixation of nitrogen it might be presumed to go on equally in either case. At the end of 3 days sugar was still present in both cases, but the nitrate had completely disappeared from Culture B. Nitrites were also completely absent.

The results calculated to dry soil were as follows :—

Culture	Date	Nitrogen by Kjeldahl	Nitrogen as ammonia	Nitrogen as nitrates (Parts per million)	Total nitrogen including that of nitrates
		Per cent.	Per cent.		Per cent.
A	Commencement ..	0·048	0·000	0·0	0·048
	After three days..	0·049	0·000	0·0	0·049
B	Commencement ..	0·048	0·000	102	0·058
	After three days..	0·057	0·000	0·0	0·057

Thus allowing for error in the Kjeldahl estimation it would appear that the whole of the nitrate added to Culture B was broken down and appears under the influence of sugar as organic nitrogen. This fact considered along with the entire absence of ammonia and nitrites points to a type of denitrification differing entirely from what is generally understood by this term, and which, excepting for the presence of sugar, takes place under conditions otherwise those usually associated with a rapid nitrification rather than denitrification.

It may here be remarked that certain other cultures in which straw (1 per cent.) was used, instead of sugar, as a medium for supplying the carbohydrate necessary for nitrogen fixation, liberally supplied with carbonate of lime, well aerated, and kept at the optimium moisture content at a temperature of 28°—30°C. showed no nitrification whatever over a period of 6 weeks. Apparently then straw acts in a similar way to sugar in this respect, though without leading to any demonstrable fixation of nitrogen.

Referring back to Fig. 2, it appears that the net loss of soil nitrogen, expressed as a percentage on initial nitrogen, was much reduced on limed field plots uncropped when phosphoric acid in addition to sugar was applied. The very marked effect of phosphoric acid on this soil especially in a basic form was described in a previous Memoir, one theory advanced for its action being an increase in soil oxidation processes.

A good deal of evidence in regard to its stimulation of soil bacterial processes has accumulated. It was constantly observed in nitrogen fixation cultures using sugar, that adding a little phosphoric acid hastened very materially the disappearance of sugar from the soil medium, at the same time by the way increasing the rate of nitrogen fixation. Thus two cultures of the same soil set up on September 6, to both of which 1 per cent. sugar was added (sodium phosphate supplying 0·01 per cent. P_2O_5 being added in one case only) showed that the sugar had totally disappeared 8 days later from the phosphated culture alone, and nitrification had set in. At the end of 7 weeks sugar was still detectable in the unphosphated soil, nitrates being consequently quite absent; while in the other case the nitrate nitrogen had reached over 32 parts per million by this time.

Measuring this increase in the rate of oxidation of sugar by the increase in evolution of carbon dioxide in a given time, by a method to be described later, it was found that the addition of 0·01 per cent. P_2O_5 as sodium phosphate led to an immediate increase of 50 per cent. in carbon dioxide evolved from a sugared soil, as against a similarly sugared but unphosphated culture, while for a period of 8 days the average increase was over 30 per cent.

Again, using soil cultures to which was added 2 per cent. of chopped cowpeas (*Vigna catjang*), instead of sugar, and measuring the evolution over a period of five weeks, it was proved that the addition of 0·01 per cent. of P_2O_5 led to an average increase of 40 per cent. carbon dioxide evolved.

Nitrogen Fixation.

In conjunction with the field work described above, laboratory studies were made of the nitrogen fixing power of this soil under varying conditions.

The plan of the experiments was as follows :—

I. Soil only.
II. Soil+sugar.
III. Soil+lime.
IV. Soil+lime+sugar.
V. Soil+lime+phosphate.
VI. Soil+lime+phosphate+sugar.

The various cultures were carried out side by side at the same temperature, 28°—30°C., and having a moisture content of 16·5—17 per cent. Where sugar was applied it was added at 0, 31 and 45 days. The soil samples for these experiments were taken from three adjoining plots : for Cultures I and II the soil of an unlimed plot was used ; for III and IV that of a plot limed two years previously ; while in the case of Cultures V and VI the plot was limed two years before and had received two dressings of sodium phosphate supplying 0·01 per cent. P_2O_5 on each occasion.

The nitrogen in each sample was estimated at the commencement, and thereafter at the end of 21, 43 and 66 days.

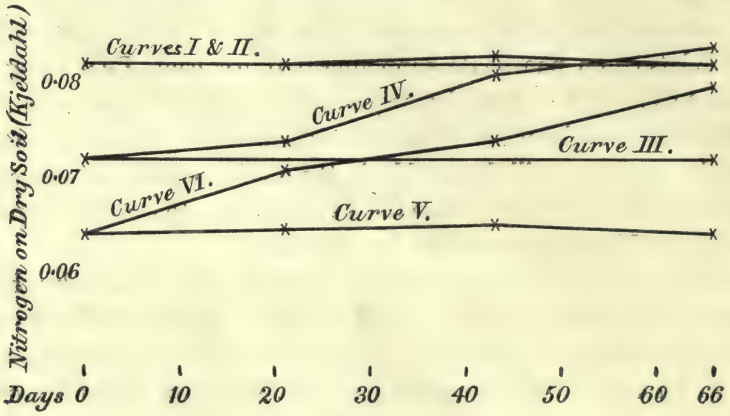

FIG. 4.

Curve I. Soil only — no fixation.
, II. Soil + sugar — no fixation.
„ III. Soil + lime — no fixation.
„ IV. Soil + lime + sugar — fixation.
„ V. Soil + lime + phosphate — no fixation.
„ VI. Soil + lime + phosphate + sugar — fixation.

The results are shown in Fig. 4 ; briefly the figures show that :—

(1) The addition of lime is necessary with this soil for nitrogen fixation ; no fixation at all took place in the unlimed soil (Curves I and II) even when sugar was used.

(2) Some carbohydrate, *e.g.*, sugar, is required; the limed soil failed to fix any nitrogen in absence of sugar, but did so when sugar was added (Curves III and IV).

(3) Phosphoric acid appears to stimulate fixation; thus the limed and sugared culture (Curve IV) showed a gain of nitrogen in 66 days equal to 16·6 per cent. of its initial nitrogen content, increased where phosphate was used (Curve VI) to 25 per cent.

Culture IV was continued for further observations, as to the effect of the cessation of sugaring, and thereafter to ascertain what limit, if any, there is to nitrogen fixation if sugar is continuously used at intervals. The result is shown in Fig. 5 up to date.

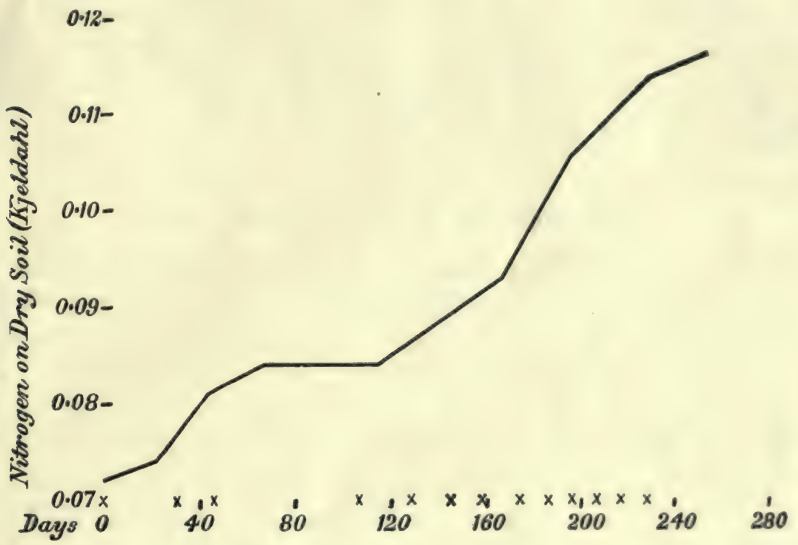

FIG. 5.
Asymbiotic nitrogen fixation.

Sugar was added, 1 per cent. regularly, as soon as it was found to be absent by the d-naphthol test. The points of such addition are indicated by crosses on the abscissa. It will be observed that so long as sugar is supplied, continuous fixation takes place. No sugar was added between the 44th and 106th days, *i.e.*, for two months, and the effect is clearly shown in a temporary cessation of fixation, resumed when sugaring re-commenced. At the time of writing this culture is still fixing nitrogen vigorously, having up to date fixed an amount of atmospheric nitrogen equal to more than 60 per cent. of its original nitrogen content.

The use of sugar as a practical aid to fixation in the field is quite out of the question, and an attempt was made to substitute straw, supplying as it does a large amount of organic matter having a rather small nitrogen content. Paddy and oat straw (dried, finely ground and sieved through 1 mm. sieve) were used in two cultures at the rate of 1 per cent. on the weight of soil, other conditions being made as favourable as possible for fixation. Thus 1 per cent. calcium carbonate was added and sodium phosphate to give 0·01 per cent. P_2O_5. Moisture was maintained at 16·5 to 17 per cent., and the temperature at 28°—30°C. The oat straw contained 0·232 per cent. nitrogen and the paddy straw 0·475 per cent. In 6 weeks no demonstrable fixation was apparent in either case. As previously remarked both these cultures were quite free of nitrates at the end of 6 weeks incubation, which seemed curious in that, excepting for the presence of the straw, all the conditions should have favoured a rapid nitrification. Straw then resembles sugar in preventing the accumulation of nitrates, but unlike the latter appears to be unable, so far as these tests go, to occasion any nitrogen fixation.

The evolution of the CO_2 from soils.

In connection with the foregoing work the evolution of CO_2 from soils of the experimental plots was studied under varying conditions. The behaviour of this soil has been a matter of field experiment and observation for the past 15 years and is well understood, and it was sought to determine amongst other things how far CO_2 evolution is a positive index of improved fertility. The method finally adopted will be readily understood from the photograph appearing as the frontispiece. A sample of the soil under experiment was thoroughly mixed, and 500 grm. placed in each of the Woulff's Bottles B and D. The aspiration was conducted in tandem to ensure equal conditions, the CO_2 evolved in B passing into Reiset A containing baryta, that from D being absorbed in Reiset C. Preliminary experiments using extra intermediate Reisets proved that CO_2 absorption was absolutely complete, under the conditions of the experiment, in Reisets A and C, no CO_2 passing over from Reiset C into Woulff's bottle B for instance.

Previous to introducing any differential treatment several aspirations were made until an equal CO_2 evolution was established in both Woulff's bottles. This done, both bottles were now emptied, and the desired differential treatment introduced in one case only. The soils were refilled into their respective bottles, and aspirations contained. Or, in case it was desired for instance to test two forms of phosphate against each other, after establishing

the preliminary equality in CO_2 evolution, one phosphatic manure was introduced into one bottle, and the other into the second one.

As previously remarked, it was observed that the addition of a little sodium phosphate to a sugared soil hastened the disappearance of the sugar very materially, incidentally increasing the rate of nitrogen fixation also, and presumably the oxidation of any organic matter would be similarly accelerated. To test this, to a given soil 2 per cent. of its weight of chopped green cowpea plants was added, and the moisture content adjusted to 16·5 per cent. Five hundred grammes of the mixture were introduced into each Woulff's bottle and aspirations made until CO_2 evolution was equalized. In one case there was then added 0·01 per cent. of phosphoric acid as sodium phosphate and the aspirations continued with the following results :—

Date	Litres of air aspirated	Duration of aspiration	Milligrams of CO_2 evolved	
			Bottle I	Bottle II
27–9–21	5	About 5 hours	66·0	63·0
28–9–21	5	„	55·0	54·0
29–9–21	Sodium phosphate added to Bottle I.			
30–9–21	5	About 5 hours	68·0	50·0
1–10–21	5	„	50·0	38·0
3–10–21	5	„	65·0	50·0
19–10–21	5	„	11·5	4·0
2–11–21	5	„	9·0	3·7

(*N.B.*—From 3-10-21 to 19-10-21 and also from 20-10-21 to 29-10-21 both Woulff's bottles stood with side tubes open, and were exhausted of any CO_2 daily by the bellows.)

It will be seen that the added phosphate increased the CO_2 evolution by 40 per cent. on the average of five aspirations continued over a period of a month.

The effect of phosphoric acid in the field has always been a marked one in its basic forms. Basic slag for instance influences cropping to a much greater extent than was superphosphate, the latter, while effective if used along with lime, being of little use without it.

The following results were obtained in a test made of CO_2 evolution using basic slag against superphosphate.

Date	Litres of air aspirated	Duration of aspiration	Milligrams of CO_2 evolved	
			Bottle I	Bottle II
27–6–22	5	About 5 hours	34	34
1–7–22	5	„	25	25
4–7–22	To Bottle I was added 0·02 per cent. P_2O_5 as basic slag. „ „ II „ „ 0·02 „ „ „ superphosphate			
7–7–22	5	About 5 hours	34	28
11–7–22	5	„	47	30
14–7–22	5	„	30	19

Here then the much greater field effect of basic slag is accompanied by an increased CO_2 evolution.

So far then the above, amongst other tests, appeared to indicate that greater fertility and increase in CO_2 formation go hand in hand. That this is not invariably so is proved by a further test using sulphate of ammonia. In the field, in absence of preliminary liming, sulphate of ammonia has shown itself to be very toxic to a variety of cropping over a period of years. A small plot to which small annual doses of this fertilizer are added has steadily become more toxic until to-day it is incapable of carrying even weeds in the rainy season, when otherwise weeds are such a terrible nuisance on this farm.

The aspiration results with sulphate of ammonia, using unlimed soil, were as follows :—

Date	Litres of air aspirated	Duration of aspiration	Milligrams of CO_2 evolved	
			Bottle I	Bottle II
18–7–22	5	About 5 hours	34	34
21–7–22	5	„	22	22
22–7–22	To Bottle I added 0·25 grm. sulphate of ammonia (=100 parts nitrogen per million of soil).			
25–7–22	5	About 5 hours	24	20
28–7–22	5	„	23	20
1–8–22	5	„	25	23
18–8–22	5	„	13	12

This result appears to indicate that for sour soils at any rate increase in CO_2 evolution is not invariably correlated with enhanced fertility. Estimations of nitrogen as nitrate made at the conclusion of the aspirations, 36 days from the commencement, showed that the soil to which was added the sulphate of ammonia contained 13·6 parts per million, while the untreated soil had 9·6 parts. Thus of the ammonia nitrogen added to Bottle I, only 4 per cent. had been nitrified in 36 days under optimum moisture conditions and temperature 28°—30°C., a much smaller amount than should be nitrified in the time and under the conditions in the case of a fertile soil.

When one considers the numberless millions of bacteria at work in a soil breaking down organic matter, etc., the various processes being carried on by distinct species requiring different conditions for their best results, it is not hard to realize that under some conditions a species or number of species of bacteria not closely associated with the processes usually considered as vital, *e.g.*, nitrification, fixation of nitrogen and so on, may swamp the more beneficial organisms. When the nitrogen content of a soil passes a certain limit the decay bacteria increase rapidly and in the struggle for existence may easily suppress other species. The use of sulphate of ammonia might conceivably lead to such a state of affairs, and would be accompanied by increased CO_2 evolution. Again, since in certain soil processes, *e.g.*, nitrification, the action of many species is more or less supplementary, one can conceive a definite optimum balance between these various organisms, and anything tending to upset this balance may limit their activities. In view of the fact that the different organisms vary in their requirements, *e.g.*, for air and moisture amount and kind of food, temperature, etc., etc., and also that they are affected unequally by changing environmental conditions, one must conclude that the bacterial equilibrium varies constantly more or less. The use of sulphate of ammonia for instance in our sour soil, where the nitrifying bacteria are already more or less embarrassed by acidity, might further upset the balance of organisms concerned in the nitrification cycle by unduly stimulating the decay bacteria. This would lead to an increased evolution of carbon dioxide and to a limitation of nitrification.

Though a great deal more work along these lines would be necessary before one could attempt to generalize, it would appear that this method of study may prove useful but that more reliable information would be likely to accrue if in all cases before aspiration, and again after aspirations are concluded, estimations of nitrate nitrogen are made.

As a method of estimating comparative CO_2 evolution, the method described leaves little to be desired and is capable of great accuracy.

General.

The work reviewed in this paper proves what very great losses of soil nitrogen may take place in a humid and hot climate with a heavy rainfall during about half the year. On uncropped plots this rate of loss is much increased by liming, and bare fallows in the rainy season are especially wasteful ; on the other hand, when cropped, the increase in vegetation due to liming tends to conserve the nitrogen. It seems probable that these losses are incurred chiefly by leaching of nitrates in drainage waters. Although the production of nitrates takes place very slowly in temperate climates, under our conditions it is relatively a much more active process, so much so that probably even on unlimed areas the loss is normally almost entirely due to leaching of nitrates.

When the land is cropped this leaching is reduced naturally, and moreover nitrification may be retarded at times by a reduction of soil moisture as a result of active transpiration. This combined with the nitrogen returned to the soil in the roots and other residues of crops may serve to explain the observed lower rate of soil nitrogen loss from cropped land. The work described provides another reason, if one were necessary, for green-cropping during the rainy season wherever this can be introduced even between standing crops when grown in lines so that the green-crop can be hoed in. By so doing not only is a good deal of waste of soil nitrogen prevented, but the crop itself adds further nitrogen gathered from the air. But apart from green-cropping, even a loose covering of grass and weeds periodically hoed in during the rains must have a conservative effect.

In regard to nitrogen fixation it appears to be absolutely essential that the soil should be limed ; phosphoric acid used periodically would seem also to be helpful. As a source of energy for the fixing bacteria sugar is pre-eminent, but, of course, out of the question for field use. Though cellulose decomposition products may serve as the necessary source of energy, so far as my experiments went the use of straw occasioned no fixation. Possibly the decomposition did not proceed sufficiently far or in the right direction under laboratory culture conditions. The conditions obtaining in the field where green-manure crops are ploughed in occasionally, and cowdung is used, and where moisture and temperature conditions vary considerably from time to time, might well result in the production of different decomposition products from those resulting in laboratory cultures.

The nitrogen position on the sugarcane rotation areas on the Jorhat Farm may perhaps have some significance in the light of the work described.

These areas have been under a four-year rotation for the past twelve years or more, embodying two years under cane (manured) and two under green manures and other cropping. They are limed every fourth year, and part of each block also receives a dressing of phosphate once in four years. At the present time the position is this: that in three cases out of four, the phosphated areas show a present average soil nitrogen content some 13 per cent. in excess of that of the otherwise similarly treated but unphosphated areas, equal to an excess of 180 lb. nitrogen per acre 0—6″. It is only fair to state that these phosphated areas have regularly returned bigger leguminous green crops in the rotation, but to balance this, other crops, *viz.*, sugarcane and oats, have removed more nitrogen year by year from these areas. It would need a much greater increase in green crops than has actually resulted to account for the whole of the present excess of nitrogen in the surface soil of the areas receiving phosphatic manure. While it cannot be claimed that this excess is due to asymbiotic nitrogen fixation, the possibility of this being the case certainly cannot be ruled out under the conditions.

Soil work both at Jorhat and in the newly opened out sugarcane tract in the Kamrup District of Assam serves to show that on opening out cultivable waste lands a more or less rapid fall of nitrogen initially must be expected. The mere cultivation of such soils which may have lain as they are for generations under jungle, adding year by year to their stores of humus, must assuredly set up a train of bacterial processes which would liberate in one form or another a good deal of nitrogen. So far there would appear to be no royal road to prevent this, but sound agricultural practice will undoubtedly prevent this process being carried too far. Apart from the use of excessive quantities of nitrogen in manure, under our conditions the adoption of a sound rotation embodying green manuring with the periodical use of lime and phosphate would appear to offer the only practical solution of the nitrogen problem. When these conditions obtain, the apparently inevitable initial fall in soil nitrogen need not occasion excessive alarm. The cane blocks on the Jorhat Farm, though now showing on the average a soil nitrogen percentage amounting to less than 70 per cent. of their initial content in spite of green-cropping and heavy direct manuring for the cane crops, produce to-day from two to three times the crops of sugarcane which it was possible to obtain when the land was newly opened out, and with less direct application of nitrogen in manures than was the case formerly

APPENDIX.

A. Chemical analysis of the Jorhat surface and sub-soils.

		Surface soil	Sub-soil
		Per cent.	Per cent.
Soluble in 26 per cent. hydrochloric acid with 12 hrs. digestion at 100°C	Phosphoric acid (P_2O_5) ..	0·025	0·020
	Potash (K_2O) ..	0·115	0·135
	Lime (CaO) ..	0·154	0·144
	Magnesia MgO) ..	0·166	0·148
Soluble in 1 per cent. citric acid with seven days digestion.	Phosphoric acid ..	0·008	0·008
	Potash ..	0·007	0·011
Loss on ignition=organic matter + combined water .		3·260	1·840
Nitrogen		0·114	0·051
Carbonic acid (CO_2)		0·009	0·007
=Calcium carbonate		0·020	0·015
Reaction		Acid	Acid

B. Mechanical analysis (ignited fractions).

		Surface soil	Sub-soil
		Per cent.	Per cent.
Fine gravel	(3 — 1 mm. diam.)	nil	nil
Coarse sand	(1 — 0·2 mm. diam.)	7·2	6·3
Fine „	(0·2 — 0·04 mm. diam.)	52·5	52·8
Coarse silt	(0·04 — 0·01 mm. diam.)	22·6	17·6
Fine „	(0·01 — 0·002 mm. diam.)	5·0	9·4
Clay	(0·002 mm. diam.)	6·6	9·9
Moisture + Loss on ignition	.. ..	5·1	3·1
		99·0	99·1

C. Moisture constants.

	Hygroscopic capacity	Maximum water-saturation capacity		Minimum water-saturation capacity		Moisture in air-dry soil
		Per cent. of water in saturated soil by weight	Per cent. of water in saturated soil by volume	Per cent. of water by weight	Per cent. of water by volume	
Surface soil..	3·10	31·9	50·5	11·2	13·9	1·30
Sub-soil ..	..	30·0	50·0	7·2	9·1	1·19

D. Acidity.

Expressed as parts quicklime (CaO) required to neutralize one million parts of air-dry soil, the acidity of the surface and sub-soils on the Jorhat Farm varies between the following limits :—

Surface soil 880—1350 parts CaO per million.

Sub-soil 650—770 parts CaO per million.

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